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Peter Saenger
Southern Cross University

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Future experiments will work towards establishment of a simple three trophic level food chain of producer-hervivore-carnivore in steady state, which can be used to investigate transfer efficiencies between trophic levels, concentration of pollutants through the food chain and other questions.

SIMPLE BROMOPHENOLS IN THE RED ALGAE AND SEAWATER

by P. SAENGER

Institute for Physiological Botany
University of Uppsala, Sweden

ABSTRACT

Using a combined GLC/MS method, 23 species of red algae representing nine orders were examined for simple brominated phenols. A total of 13 brominated phenols were identified (MS, NMR and IR) from the Gigartinales, Cryptonemiales, Bonnemaisonailes and Ceramiales. Seawater from the *Polysiphonia brodiaei*-zone also contained two bromophenols. The taxonomic and ecological implications are discussed.

A number of bromine-containing organic compounds have been described from the Rhodomelaceae (Rhodophycophyta); these include simple bromophenols (AUGIER and COLIN, 1939; SAITO and ANDO, 1955; HODGKIN et al., 1966; CRAIGIE and GRUENIG, 1967; SAENGER, 1970; GLOMBITZA and STOFFELEN, 1972; STOFFELEN et al., 1972), brominated heterocyclic compounds (IRIE et al., 1969; FUKAZAWA et al., 1972; SIMS et al., 1972) and the as yet poorly known red pigment floridorubin (SAENGER, 1970). No organic bromo-compounds have been isolated from red algae not belonging to the Rhodomelaceae, although KYLIN (1929) suspected covalently-bound bromine in *Antithamnion, Antithamnionella, Ceramium, Trailliella* and *Bonnemaisonia*.

The occurrence and distribution of simple bromophenols have been investigated by the colour reaction with $FeCl_3$ (AUGIER, 1953) and their chromatographic behaviour (PEGUY, 1964). However since the number of red algae investigated is small, 23 species belonging to the various families were investigated to determine a more exact distribution of these compounds.

A list of the algae examined and the relevant collection data are provided in Table 1. The algal material was briefly rinsed in freshwater and over dried at 450°C for 48 hours. Once dried, the material was pulverised using a mortar and pestle and the powder subsequently treated as set out in Figure 1.

The bromophenols were examined by a combined gas chromatography-mass spectroscopy technique as described by PEDERSEN, FRIES and SAENGER (in press). Trimethyl-silyl derivatives were prepared and the analysis carried out with a Varian Mat III (Gnom) mass spectrometer coupled to a Varian Aerograph Gas Chromatograph equipped with a 4 foot, 2 mm internal diameter silanized glass column. Helium was used as carrier gas at a flow rate of 25 ml/min.

NMR analyses were carried out in deuterated acetonitrile on a Varian A-60 spectrometer with tetramethylsilane as an internal standard. IR spectra were made on a KBr disc using a Perkin-Elmer IR Spectrophotometer. Several spray reagents were used including 3% aqueous $FeCl_3$ and 0.1 M $Na_2MoO_4 2H_2O$, a spray specific for orthy-dihydroxy compounds.

Analyses of seawater samples were carried out at Kristineberg Zoological Station. Seawater was collected from the *Polysiphonia brodiaei*-zone, filtered through Munktell paper (A3-90-700) and reduced from 6 litres to 1 by rotary evaporation. It was subsequently made to 0.05 N NCl and heated to 70°C for 0.5 hours to hydrolyze any ester sulphates. The seawater was then shaken with ethyl acetate and the process repeated seven times. After removing any water, the ethyl acetate extracts were combined and reduced in volume to about 5 ml. After freeze-drying, the analytical technique as previously described was used.

With the exclusion of the tri-bromophenols which will be discussed elsewhere, a total of 13 simple mono- and di-bromophenols were found and their distribution is given in Tables 1 and 2. Of these compounds 5 (I, II, III, VIII and XI) appear to be artefacts produced during the extraction procedure; depending on the extraction medium (methanol or ethanol) methyl- or ethyl-ethers were observed while with the non-aqueous medium (ethyl acetate) these ethers were absent. In *P. brodiaei* the bromophenol content was 0.5% of the dried weight while in the non-rhodomelaceous algae it constituted less than 0.1% of the dried weight.

Analysis of the *P. brodiaei*-zone seawater yielded two bromophenols (IX and XI) in addition to a number of organic acids.

(i) Taxonomic

The known distribution of the various organic bromo-compounds is shown in Table 2. The ability to brominate phenolic compounds appears to have evolved independently in several of the algal groups and since this ability is restricted to what are generally regarded as the more advanced algal groups, it is apparently of recent origin. Of particular interest in this respect is the absence of bromo-compounds from the Dasyaceae and the Delesserieae. The occurrence of a variety of bromophenols in *Falkenbergia* and *Trailliella* is surprising in view of their inclusion in the Nemalionales on morphological and life-history data (KYLIN, 1956). Similarly the absence of simple bromophenols in the Laurencieae is in sharp contrast with the pattern of the other groups so far examined in the Rhodomelaceae. The occurrences of heterocyclic bromo-compounds in the Laurencieae further serve to distinguish this group. The occurrence of tri-bromophenols in the apparently distantly related Cryptonemiales, Bonnemaisoniales and Amansieae is puzzling and requires wider confirmation.

(ii) Ecological

The physiological role of the bromophenols is uncertain. CRAIGIE and GRUENIG (1967) postulated that they are involved in the regulation of endo- and epiphytes, since a number of bromophenols (including compound IX) were found to be toxic to several unicellular marine algae at levels below 0.3 mmol/1 (McLACHLAN and CRAIGIE, 1966). These authors found that the antialgal activity was correlated with the isomerism of the hydroxyl groups and all ortho dihydroxy compounds including the naturally occurring algal phenols were especially toxic. They concluded however that the naturally occurring phenols probably have little general ecological significance since the species in which they are found are restricted to relatively narrow ecological ranges. But since it is now known that these compounds may be excreted into the seawater, more widespread ecological effects can be expected. Because of the sensitivity of unicellular marine algae to bromophenols, measurements of primary productivity conducted in coastal waters may be influenced by bromophenols excreted from some of the macroalgae.

FRIES (unpublished) has shown that in axenic culture in artificial seawater, bromophenols may have stimulating

effects on some macroscopic algae at 0.2 mmol/1 while others show inhibition at 0.05 mmol/1. All species examined were inhibited by bromophenols above 0.2 mmol/1. PROVASOLI (1969) showed that when *Ulva lactuca* is grown without the natural microflora, it loses its leafy morphology and becomes a colony of uniseriate branching filaments. Several phenolic compounds including the bromophenol (IX) restrored the normal morphology. The bromophenol (IX) was however not active in restoring normal growth to *Monostroma oxyspermum.*

Other evidence for the ecological involvement of simple bromophenols can be seen in that many species of algae rich in bromophnols, occur in almost pure zones, are relatively free of epiphytes or are epiphytized by only certain species. This suggests a chemical regulatory mechanism is involved and it may conceivably rely on the antagonism or tolerance towards excreted bromophenols.

ACKNOWLEDGEMENT

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FIGURE 1

Flow diagram of method used for analyses of simple brominated phenols.

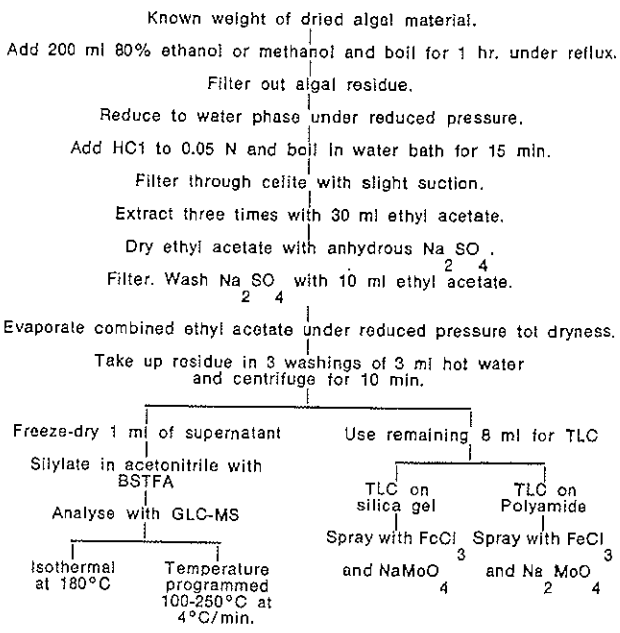

TABLE 1

List of the red algae investigated, the source of the material and the bromophenols found. (K – collected near the Kristineberg Zoological Station; I – collected by scuba diving at Islandsberg, south of the Station; S – collected by dredging at Smedjan, west of the Station; M – collected near Banyuls-sur-Mer, Mediterranean Sea; L – cultivated axenically in Provasoli's enriched seawater.)

Species and Source		Bromophenols present
Porphyra umbilicalis (L.) Kuetz.	I	—
Rhodosorus marinus Geitler	L	—
Asterocytis ramosa (Thwait.) Gobi et Schm.	L	—
Goniotrichum alsidii (Zanard.) Home	L	—
Nemalion multifidum (Weber et Mohr) J. Ag.	K	—
Rhodymenia palmata (L.) Grev.	I	—
Cystocloniun purpureum (Huds.) Batt.	S	—
Dilsea carnosa (Schmidel) Kuntze	S	—
Corallina officinalis L.	K	IX
Trailliella intricata Batt.	K	A, C
Falkenbergia rufolanosa (Harv.) Schmitz	M	B
Polysiphonia brodiaei (Dillw.) Grev.	K	IV, V, VIII, IX
Polysiphonia urceolata (Dillw.) Grev.	I	IV, V, VII, IX
Polysiphonia nigrescens (Huds.) Grev.	I	IV, VI, VIII, IX
Rhodomela confervoides (Huds.) Silva	I	VI, VII, VIII, IX
Odonthalia dentata (L.) Lyngb.	I	I, II, III, IV, V, VI, VII, VIII, IX, XI
Laurencia pinnatifida (Huds.) Lamour.	I	—
Heterosiphonia plumosa (Ellis) Batt.	S	—
Dasya baillouviana (Gmel.) Mont.	K	—
Phycodris rubens (Huds.) Batt.	I	VIII
Delesseria sanguinea (Huds.) Lamour.	I	—
Antithamnion plumula (Ellis) Thur.	S	VIII, IX
Ceramium rubrum (Huds.) Ag.	S	VIII, IX
Polysiphonia brodiaei – seawater	K	IX, XI

TABLE 2

Distribution of the various classes of organic bromocompounds in the red algae.

Group	Simple bromophenols C Br,Br 7-9 2	C Br 7-9 3	Heterocyclic C Br,Br 14-17 2	C Br Floridorubin 32 x
Porphyridiales	-	-	-	-
Bangiales	-	-	-	-
Goniotrichales	-	-	-	-
Nemalionales	-	-	-	-
Rhodymeniales	-	-	-	-
Gigartinales	+	-	-	-
Cryptonemiales	+	+	-	-
Bonnemaisoniales	+	+	-	-
Ceramiales				
Rhodomelaceae				
Polysiphonieae	+	-	-	+
Amansieae	+	+	-	+
Rhodomeleae	+	-	-	+
Laurencieae	-	-	+	-
Delesseriaceae				
Delesserieae	-	-	-	-
Nitophylleae	+	-	-	-
Dasyaceae	-	-	-	-
Ceramiaceae	+	-	-	-

POPULATION ESTIMATION OF MARINE DECAPODS

by G. R. MORGAN

The most crucial assumption that any mark-recapture method for calculating population estimates makes is that all live animals in the population are equally at risk to recapture. Information on the validity of this assumption for a population of the western rock lobster *(Panulirus cygnus) has been collected recently by comparison of* pot-caught and diving-caught animals. It was found that previously pot-marked rock lobsters had a higher probability of recapture by pots than unmarked rock lobsters (tables 1 and 2), although there appeared to be no selectivity by pots for types of marks (table 3). This results in little or no bias in the estimation of the number of marked animals in the population, but an underestimate of population size.

This differential response of marked and unmarked rock lobsters to a baited pot can be a result of an initial distribution of probability of capture over the population, a situation which may exist in many other Decapods which are sampled by baited traps (e.g., in marron, *Cherax tenuimanus* as shown by Morrissy, 1973). Hence, such a distribution leads to a negative bias in the estimated population size.

The degree of bias can be plotted as a function of marking intensity. Such a diagram shows that bias is reduced by:

1 high marking intensity, or exploitation rate; and

2 multiple marking before the recapture operation or, in general, by marking as high a proprotion of the population as possible.

TABLE 1

Marked rock lobsters, previously caught and released from pots, which were taken by diving and by pots during December 1970, December 1971, June and October 1972, expressed as a proportion of the total (marked and unmarked) captures by each method each month.

Month	Pot Caught (P) Recaptures	Div'g Caught (D) Recaptures	P / D
December 1970	808/2377 = 0.34	104/435 = 0.24	1.4
December 1971	671/3195 = 0.21	111/740 = 0.15	1.4
June 1972	379/599 = 0.63	263/774 = 0.34	1.9
October 1972	280/380 = 0.74	151/325 = 0.47	1.6

TABLE 2

Percentage of Diving-marked and Pot-marked rock lobsters which were subsequently recaptured by pots.

Month of Release	% Recaptures of Diving-marked animals	% Recaptures of Pot-marked animals
December 1970	17.2%	25.8%
December 1971	12.8%	21.1%
June 1972	31.0%	52.9%
October 1972	35.6%	47.9%

TABLE 3

Percentage composition of marked animals, previously caught and released from pots, which were recaptured by diving or by pots in December 1970, December 1971, June 1972, and October 1972.

December 1970

Last marked in:-	Pot Caught	Diving Caught
November	13.9%	15.4%
September	10.2%	5.8%
August	14.3%	11.5%
July	6.4%	7.7%
June	11.0%	8.7%
April	18.0%	22.1%
Earlier than April	26.1%	28.9%
Total	99.9%	100.1%
Total (Nos.)	368	104

October 1972

Last marked in:-	Pot Caught	Diving Caught
August	28.0%	31.8%
July	18.4%	13.3%
June	8.4%	8.0%
May	6.2%	7.3%
April	14.2%	15.9%
March	16.1%	15.9%
Earlier than March	8.7%	8.0%
	100.0%	100.2%
	270	151

December 1971

Last marked in:-	Pot Caught	Diving Caught
Nov. 1971	14.7%	12.8%
October	10.1%	9.0%
September	12.5%	13.5%
August	13.1%	11.0%
July	13.1%	11.4%
June	6.2%	7.9%
Earlier than June	30.2%	34.5%
Total	99.9%	100.1%
Total (Nos.)	671	111

June 1972

Last marked in:-	Pot Caught	Diving Caught
May 1972	26.0%	24.1%
April	16.1%	17.7%
March	21.4%	14.2%
February	11.0%	10.0%
January	3.6%	9.0%
Dec. 1971	4.0%	8.9%
Earlier than December	18.0%	16.4%
	100.1%	100.3%
	379	263